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CULTIVATION OF *BACTERIUM TULARENSE* ON THREE ADDITIONAL MEDIUMS NEW TO THIS ORGANISM.¹

By EDWARD FRANCIS, Surgeon, United States Public Health Service.

The writer now reports the cultivation of *Bacterium tularense* on (1) beef infusion agar containing 0.02 per cent of an amino-acid (cystine), (2) beef infusion agar plus a piece of fresh sterile rabbit spleen, and (3) Loeffler's blood serum coagulated at 70° C.

The mediums here reported were used for the subcultivation of strains of *Bacterium tularense* which had had their original isolations from animals 12 months previously and had been carried continuously during the year on artificial mediums other than egg yolk, except that about 8 months previously they were carried for one or two passages through guinea pigs.

The strains used for this cultural work were human strains of "J", "G", and "S" and ground squirrel strain "S. F.", which were the subject of previous cultural studies.²

COMPOSITION OF MEDIUMS.

(1) *Cystine agar*.—Beef infusion agar containing 1 per cent peptone and 1 per cent agar adjusted to a reaction having a p_H of 7.6 is kept on hand in stock. When needed, there is added to the stock agar 0.02 per cent of cystine, and this is placed for 15 minutes at the temperature of streaming steam in an Arnold sterilizer to melt the agar and to sterilize the cystine, after which the medium is tubed, slanted, and incubated 24 hours to insure sterility.

(2) *Plain agar plus a piece of fresh sterile rabbit spleen*.—The spleen is removed from a healthy rabbit and under sterile precautions is cut into pieces of about 3 mm. diameter. One piece is rubbed on the slanted surface of each beef infusion agar slant and the piece of spleen is left remaining on the surface of each slant just above the water of condensation; one piece of spleen is planted as a control into a

¹ Eight articles, including the present article on tularæmia, which appeared in Public Health Reports, vol. 36, 1921, and vol. 37, 1922, will be combined and printed in pamphlet form as Hygienic Laboratory Bulletin No. 130. For a summary of these articles see "Tularæmia: A New Disease of Man." By Edward Francis, Jour. Am. Med. Assoc., vol. 78, 1922, pp. 1015-1018.

² Cultivation of *Bacterium tularense* on mediums new to this organism. By Edward Francis, Surgeon, United States Public Health Service. Public Health Reports, vol. 37, No. 3, Jan. 20, 1922, pp. 102-115.

fermentation tube containing glucose beef infusion broth. The tubes are incubated 24 to 48 hours, and, if sterile, are ready for inoculation.

CULTIVATION ON CYSTINE AGAR.

Cultures grown upon cystine agar are now in their fourteenth consecutive generation, having been carried over every 48 hours. Growth in the fifth generation was rubbed on the shaved abraded skin of the abdomen of a guinea pig, causing its death on the sixth day with typical lesions of tularæmia. At the moment of death the spleen was removed and, under sterile precautions, was seared and cut into pieces about 3 mm. in diameter. One piece was planted on the slanted surface of each of three cystine agar tubes, two plain agar tubes, and two serum glucose agar tubes by rubbing the tissue over the surface of the medium and then allowing it to remain just above the water of condensation. One piece was planted into each of two fermentation tubes containing glucose beef infusion broth as a control. The heart's blood was planted on cystine agar slants, plain agar slants, and into fermentation tubes containing glucose bouillon broth. All tubes were incubated at 37° C. The cultures on the infected guinea pig's spleen and heart's blood will now be discussed.

(1) *Growth from guinea pig's spleen.*—Growth appeared on the third day on all seven of the tubes of slanted medium planted with infected spleen, but the fermentation tubes are still clear on the thirteenth day. The growth on the seven tubes had the morphology of *Bacterium tularensis*, was Gram negative, and was subcultured as follows: Cystine agar growth to cystine agar slants, plain agar growth to plain agar slants plus a piece of fresh sterile rabbit spleen, serum glucose agar growth to serum glucose agar slants, and all seven to control tubes of plain agar slants and glucose fermentation tubes.

The controls are all negative at the end of ten days, but all other tubes showed good growth at the end of 24 hours, which has been subcultured daily, each to its own kind of medium. These subcultures in the fifth generation were rubbed on the shaved abraded skin of the abdomen of guinea pigs on April 25, causing acute death with typical lesions of tularæmia.

(2) *Growth from guinea pig's heart's blood.*—Growth appeared on the sixth day on the cystine agar slants planted with the guinea pig's heart's blood, and by the eighth day growth was abundant. Daily subcultivation on cystine agar and plain agar give good growth on the former but no growth on the latter. The fermentation tubes planted with the guinea pig's heart's blood are sterile on the thirteenth day.

CONCLUSION.

Cultures of *Bacterium tularensis* of human and ground-squirrel origin which have been carried one year on artificial mediums other

than coagulated egg yolk grow well on (1) cystine agar, (2) plain agar plus a piece of fresh sterile rabbit spleen, and (3) Loeffler's blood serum coagulated at 70° C. The same cultures fail to show growth on plain agar and in fermentation tubes containing beef infusion broth.

Cultures in the fifth generation on these special mediums caused acute death with typical lesions of tularæmia in guinea pigs from which *Bactreium tularense* was cultured on the same mediums; these latter cultures in the fifth generation caused acute death in guinea pigs with typical lesions of tularæmia.

NOTE.—Old cultures of gonococcus and *B. diphtheriæ* grow abundantly on cystine agar.

THE COMPARATIVE ANTISCORBUTIC VALUES OF MILK.

By J. M. JOHNSON, Chemist, and C. W. HOOPER, Pathologic Physiologist, United States Public Health Service.

Review of the Literature.

Bolle¹ found that guinea pigs fed on boiled milk developed scurvy, while those given raw milk did not. Bartenstein² disagreed with Bolle.

Frøehlich³ found that by feeding guinea pigs on raw cow's milk alone, the pigs showed an extraordinary brittleness of bones, a symptom which in the disease picture of infantile scurvy plays a great rôle, while the other symptoms of this disease—the typical hemorrhages, the tooth affections, and the specific bone changes—do not enter. When fed oats alone guinea pigs always developed scurvy with brittle bones and definite symptoms. But when fed a combination of oats and raw milk the animals were maintained in normal health. The addition of raw milk not only prevented scurvy, but the combination also prevented brittleness of bones. Heated milk did not prevent scurvy.

Hart⁴ fed monkeys on canned condensed milk, and they became typically scorbutic. Funk⁵ states that heated milk induces scurvy in infants. Hess and Fish⁶ found that in several cases infantile scurvy occurred as a result of feeding milk which had been pasteurized at 63° C. for only 30 minutes. Janet Lane-Claypon⁷ concluded that animals and infants develop better on milk of their own

¹ Zeitschr. f. diät. u. physik. Therapie, 6, 354 (1903).

² Jahrb. f. Kinderh., 61, 6 (1905).

³ Zeitschr. f. Hyg. u. Infektionskr., 72, 155 (1912).

⁴ Arch. f. Path. Anat. u. Physiol. u. f. klin. Med. (Virchow) 208, 367 (1912).

⁵ Ergeb. d. Physiol., 13, 125 (1913).

⁶ Am. Jour. Dis. Child., 8, 385 (1914).

⁷ Ergebn. d. Inn. Med. u. Kinderh., 1913, 635; Report to the Local Government Board, London (1912), N. S. No. 63, 1219.